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Little loss of 2,4,5-T was evident in tropical soils in the first two months after addition of the herbicide, but the rate of disappearance then increased with time. Little disappearance was evident in four months in sterile soil. The production of $^{14}CO_2$ from ^{14}C-ring-labeled 2,4,5-T was detected in one week in two tropical soils, but two months was required for significant $^{14}CO_2$ production in two other tropical soils. In the decomposition of 2,4,5-T in soil, 2,4,5-trichlorophenol appeared and then

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disappeared. A bacterial culture destroyed about 70% of the 2,4,5-T in a medium in 80 h, and nearly 60% of the herbicide that was metabolized was recovered as 2,4,5-trichlorophenol. The bacterium did not use the trichlorophenol as a carbon source, and it did not release $^{14}CO_2$ from ^{14}C-2,4,5-trichlorophenol or ^{14}C-2,4,5-T. 2,4,5-Trichlorophenol was converted by microorganisms in soil suspensions to 3,5-dichlorocatechol, 4-chlorocatechol, succinate, and probably cis cis-2,4-dichloromuconate, 2-chloro-4-carboxymethylene-but-2-enolide, and chlorosuccinate.

Carbaryl, Malathion, Diazinon, Dursban, Dichlorvos, and 2,4,-D did not retard oxygen consumption by sewage at chemical concentrations ranging from 0.1 to 100 µg/ml. Baygon at 10 and 100 µg/ml and Ficam at 1 and 10 µg/ml slowed oxygen consumption during the decomposition of sewage. Of the eight pesticides, only 14 µg/ml Carbaryl exerted a detrimental influence on generation time of sewage-derived nitrifiers. Baygon, Ficam, and 2,4-D had no effect at either 1.4 or 14 µg/ml. Malathion, Diazinon, Dursban, and Dichlorvos stimulated nitrification at a concentration of 10 µg/ml, but they had no effect at 1 µg/ml.

Enrichments were obtained able to deplete O_2 at the expense of Carbaryl, Ficam, Dichlorvos, Baygon, Malathion, and Diazinon. An enrichment containing principally two pseudomonads and a bacillus degraded Dichlorvos, and metabolites were noted by TLC, GLC, and mass spectral analyses. The presence of dichloroethanol was confirmed, and evidence was obtained for the presence of 1, 1-dichloroethyl acetate. Inorganic phosphate was formed in increasing amounts. Evidence for biological demethylation was obtained.

OFFICE OF NAVAL RESEARCH

Contract N0001478C-C044

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FINAL REPORT

Microbial Degradation of Pesticides

by

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30 November 1981

MAJOR ACCOMPLISHMENTS

Phenoxy herbicides such as 2,4-D and 2,4,5-T are among the most commonly used chemicals for selective weed control and for defoliation. This study focused initially on the persistence of 2,4-D and 2,4,5-T in previously unstudied environments and on the role of microorganisms in the fate and degradation of these pesticides.

Fifty-two bacteria isolated from sewage, temperate soil, and various tropical soils were tested for their ability to attack 2,4-D and 2,4,5-T. Fourteen caused the disappearance of 35 to 100 percent of the 2,4-D, and nine brought about the destruction of 20 to 100 percent of the 2,4,5-T. None of the organisms could use 2,4-D or 2,4,5-T as a sole source of carbon. Degradation of 2,4-D and phenoxyacetic acid in nonsterile sewage and a tropical soil was greatly enhanced by pretreating the sewage and soil with these compounds, suggesting the selection for organisms capable of attacking 2,4-D and phenoxyacetic acid. Cell yields of the three most active 2,4,5-T degraders in a medium with glucose, glycerol, and sodium succinate and in a benzoate-supplemented medium with and without 2,4,5-T did not differ, suggesting cometabolic attack. Resting cell suspensions of nine of the isolates cleaved chlorine from the 2,4,5-T molecule while metabolizing more than 40 percent of the 2,4,5-T, suggesting ring cleavage of the herbicide. Eight isolates produced chlorinated phenol from 2,4,5-T. Studies of the respiratory activity of three isolates also suggested ring cleavage of 2,4,5-T. The microbial role in degradation of 2,4,5-T in soils was further shown by use of ^{14}C-ring labeled 2,4,5-T.

Little loss of unlabeled 2,4,5-T was evident in four tropical soils in the first two months after addition of the herbicide, but the rate of disappearance then increased with time. Little disappearance was evident in four months in gamma-irradiated soil. The production of $^{14}CO_2$ from ^{14}C-ring-labeled 2,4,5-T was detected in one week in two tropical soils, but two months was required for significant $^{14}CO_2$ production in two other tropical soils.

In the decomposition of 2,4,5-T in soil and soil suspension, 2,4,5-trichlorophenol appeared and then disappeared. No 2,4,5 -T decomposition was evident in soil or soil suspension sterilized by gamma irradiation. A bacterial culture destroyed about 70 percent of the 2,4,5-T added to a glucose-inorganic salts medium in 80 h, and nearly 60 percent of the herbicide that was metabolized was recovered as 2,4,5-trichlorophenol. The bacterium did not use the trichlorophenol as a carbon source, and it did not release $^{14}CO_2$ from ^{14}C-2,4,5-trichlorophenol or ^{14}C-2,4,5-T. Soil suspensions converted 8 percent of labeled 2,4,5-T and 40 percent of labeled 2,4,5- trichlorophenol to $^{14}CO_2$ in 25 days. 2,4,5-Trichlorophenol was converted by microorganisms in the soil suspensions to products that were identified as 3,5-dichlorocatechol, 4-chlorocatechol, and succinate by gas chromatography and mass spectrometry and to products that were tentatively identified as cis cis-2,4-dichloromuconate, 2-chloro-4-carboxymethylene-but-2-enolide, and chlorosuccinate by gas and thin-layer chromatography. Based on these results, a pathway of 2,4,5-T decomposition was proposed.

As federal regulations restricted the use or resulted in an outright ban of organochlorine insecticides, other pesticides were introduced to take their place. Among these are the organophosphorus and carbamate insecticides. Assessments of the toxicity and fate of many of these chemicals in soils and in vitro were made as part of the process of registration. However, their effect when introduced into sanitary sewer facilities have not been widely studied. For this reason, eight pesticides were investigated using the BOD method with a sewage inoculum. Six of the eight chemicals (Carbaryl, Malathion, Diazinon, Dursban, Dichlorvos, and 2,4,-D) did not retard oxygen consumption by a 0.33 percent sewage inoculum in a one-week test period at concentrations ranging from 0.1 to 100 µg/ml of active ingredient. Baygon at 10 and 100 µg/ml was toxic in the test period. Ficam at 1 and 10 g/ml also slowed oxygen consumption during the decomposition of sewage as compared to a control with no pesticide.

The toxicity of these same pesticides to nitrification was assessed by measuring the effect of low concentrations of the chemicals on nitrate formation. Of the eight pesticides studied, only 14 µg/ml Carbaryl exerted a detrimental influence on generation time of sewage-derived nitrifiers as determined from the logarithmic rates of nitrate formation with and without pesticides. Other carbamates, Baygon and Ficam, and the herbicide, 2,4-D, had no effect at either 1.4 or 14 µg/ml. Four organophospate insecticides---Malathion, Diazinon, Dursban, and Dichlorvos--stimulated nitrification at a concentration of 10 µg/ml, but they had no effect at 1 µg/ml.

The BOD method was also used to test the biodegradation of the pesticides with each chemical supplied as sole carbon source. Enrichments were obtained able to deplete O_2 at the expense of Carbaryl, Ficam, Malathion, and Diazinon, but Baygon, Dichlorvos, and 2,4-D were resistant to breakdown as tested by this procedure. Addition of vitamins to the medium enabled enrichments for microorganisms acting on Baygon and Dichlorvos to proceed. The extent of O_2 depletion may depend on the accessibility of oxidizable portions of the molecule to bacterial attack. The failure to observe degradability of others of these compounds may be a result of the use of a short incubation period, the need for a second carbon source if the compounds are cometabolized or the absence of growth factors.

Full details of the work described above are given in the reports submitted to ONR in 1978, 1979, and 1980.

The following information represents the accomplishments in FY 1981.

ABSTRACT

The pathway of degradation of one organophosphorus insecticide, Dichlorvos, was studied in detail. A microbial enrichment containing principally two pseudomonads and a bacillus, when incubated in a salts

solution containing a vitamin supplement and 250 µg/ml Dichlorvos as sole carbon source, could partially degrade the insecticide. At the end of 7 days incubation, much of the parent molecule was still present but biologically formed metabolites were also noted by TLC, GLC, and mass spectral analyses. The presence of dichloroethanol was confirmed, and evidence was also obtained for the presence of 1,1-dichloroethyl acetate, suggesting a mechanism of cleavage whereby the dichlorovinyl leaving group of the molecule is cleaved from the parent molecule. Whether cleavage was chemical, biological or both was not determined. Inorganic phosphate was formed in increasing amounts in a 4-day incubation period, especially in treatments containing a second more utilizable carbon source, implying a cometabolic route for the degradation of Dichlorvos. Evidence for biological demethylation was also obtained. The results provide evidence for a microbial role in the transformation in conjunction with chemical hydrolysis of Dichlorvos.

INTRODUCTION

Where the extensive use of pesticides creates a risk of large-scale contamination of surface waters, aquifers, or sewage, knowledge of the effects of pesticides on microbial processes and the fate of these chemicals becomes important. The shift in recent years from chlorinated to organophosphorus pesticides in agricultural and domestic applications has provided impetus for studies assessing the toxic effects of the latter on microbial processes of environmental importance: the organophosphate insecticides are favored because of their high insecticidal activity and relatively short persistence in the environment. One such insecticide, dichlorvos (2,2-dichlorovinyl O,O-dimethyl phosphate, DDVP, Vapona) has been shown to have little toxicity to microbial activity in sewage (Lieberman and Alexander, 1981), lake microorganisms (Murry and Guthrie, (1980), and poultry waste digester effluent slurries (Ballington et al, 1978) at 100 µg/ml or less, concentrations which are remotely likely to occur, except for an accidental spillage. As a rule, concentrations below 10 µg/ml are evident in the environment.

Regardless of the level of contamination, the introduction of such chemicals into natural environments raises the question of their chemical and biological fate. Alexander (1981) proposed type reactions for biological transformations of many classes of chemicals of environmental concern. The microbial cleavage of alkyl and aryl phosphate esters, in particular, has been shown to occur in mixed and axenic cultures, as well as in enzyme preparations derived from microorganisms (Rosenberg and Alexander, 1979). An overview of all biological and chemical transformations specific to organophosphorus pesticide can be found in Eto (1974).

The electrophilic character of the phosphorus atom generally predisposes the molecule to break by reaction at that site, whether by chemical hydrolysis or enzymatically. This cleavage most often imparts water solubility to the phosphorus-containing moiety. The leaving group, or other part of the molecule, can then be transformed further either biologically or chemically (El Beit, 1978; Faust and Gomaa, 1972; Kearney and Helling, 1969).

Although bacteria are known to carry out all the above type-reactions and organisms have been identified which can cause disappearance of the organophosphorus parent molecules (Rosenberg and Alexander, 1979), including dichlorvos (Lamoreaux and Newland, 1978; Boush and Matsumura, 1967), the actual pathway of bacterial breakdown of dichlorvos has not been shown. Because an understanding of microbial breakdown of pesticides is important practically, the present investigation was undertaken to define this pathway.

METHODS

Microbial enrichment. To obtain a microbial enrichment capable of metabolizing dichlorvos or its products, the BOD (biological oxygen demand) test method (Standard Methods, 1975) was used. Domestic sewage (0.33%, vol/ vol) was inoculated into standard BOD diluent amended to contain 100 µg/ml of dichlorvos (98%, Shell Chemical Co., San Ramon, CA) and incubated at room temperature until the dissolved oxygen level was 1-15% of that initially present, usually 5 to 7 days; oxygen depletion resulted from the oxidation of the sewage organic matter. Cells from this preparation were collected by centrifugation, but subsequently, they were found to be unable to utilize dichlorvos as a sole carbon source. When cell numbers were increased by growth in a growth medium amended with dichlorvos, 100 µg/ml peptone (Difco Laboratories, Detroit, MI) and a complete vitamin supplement, a washed mixed cell suspension from this enrichment could then deplete over 95% of the available O_2 in 5 days with 250 µg/ml dichlorvos as sole carbon source. Thereafter, the medium for maintenance of this bacterial enrichment, designated Ve, contained 0.1 g of NH_4NO_3, 0.4g KNO_3, 25 mg NaCl, 0.2g $MgSO_4 \cdot 7\ H_2O$, 2.5 mg $CaSO_4 \cdot 2\ H_2O$, 2.5 mg $FeSO_4 \cdot 7\ H_2O$, 1.60 g of K_2HPO_4, 0.4 g of KH_2PO_4, 20 µg each of biotin and folic acid, 100 µg of pyridoxine HCl, 50 µg each of riboflavin, thiamine HCl, nicotinic acid, pantothenic acid, para-aminobenzoic acid and thioctic acid, and 1 µg of vitamin B12 per liter of distilled water. The final pH after autoclaving was 7.1. The vitamin supplement provided only 0.22 µg/ml of carbon, which was insufficient to account for the amount of O_2 depleted. The Ve enrichment was thereafter maintained by harvesting cells monthly and reinoculating fresh medium containing vitamins and 250 µg/ml dichlorvos. In preparation for subsequent experiments, 150 mg of disodium succinate salt (Eastman Organic Chemicals, Rochester, NY) and 150 mg of glucose (Difco Laboratories, Detroit, MI) were added per liter of bacterial salts solution to stimulate microbial growth. A turbid cell suspension was then washed 3 times to remove residual carbon prior to inoculation into experimental treatments.

The dominant organisms were isolated from Ve. Two gram-negative rods were identified using the Analytical Profile Index (Analytab Products, Plainview, NY) as Pseudomonas aeruginosa and Pseudomonas sp. A third common organism, a gram-positive rod with spores, was tentatively identified as Bacillus sp. None of the three could individually cause O_2 depletion with 250 µg/ml dichlorvos as sole carbon source in BOD tests, even upon inoculation of greater than 10^7 cells/ml.

Experimental design and analyses. To distinquish between biological and chemical products, three treatments were prepared. Treatment 1 contained salts solution plus vitamins, 250 µg/ml dichlorvos, and more than 10^7 cells/ml. Treatment 2 contained 250 µg/ml dichlorvos alone. Treatment 3 contained microbial cells alone. Other control treatments included autoclaved cells with dichlorvos, poisoned cells (antibiotic, $HgCl_2$, or KCN) with dichlorvos, no cells or chemical. All treatments were incubated at 29°±1°C on a rotary shaker for 7 days. At the end of the incubation period, the contents of each flask were extracted with ether as follows. NaCl (2%) was dissolved in each flask. Anhydrous ethyl ether (Mallinckrodt,

Paris, Ky) was then added (25-50% of the total volume), and the liquid was mixed vigorously. The upper ether layer was collected with a separatory funnel. This procedure was carried out three times, and the ether fractions were pooled and designated the "neutral" fraction. The remaining aqueous solution was acidified to pH2 with H_2SO_4 and extracted again as above. This fraction was called the "acidic" fraction. The ether fractions were concentrated at 25°C with a rotary evaporator (Buchi Labs, Technik AG, West Germany) and dried over anhydrous Na_2SO_4 for analysis by both thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

Ten to 30 µl of each concentrated ether fraction was spotted on analytical silica gel TLC plates (Eastman Kodak Co., Rochester, NY; J. T. Baker Chemical Co., Phillipsburg, NJ) and developed in a solvent system containing petroleum ether (Mallinckrodt), 1-propanol (Fisher Scientic Co., Rochester, NY), acetic acid (Mallinckrodt), and chloroform (Mallinckrodt) 80:20:10:5). For identification, 4 to 6 ml of either neutral or acidic ether fraction was banded horizontally across a preparative glass silica gel TLC plate (Whatman, Inc., Waters Associates) and developed as above. The parent molecule and chlorinated metabolites were visualized by spraying the plates with a silver nitrate-hydrogen peroxide spray reagent (no. 232. Stahl, 1969) followed by UV irradiation for 10 to 15 min. Metabolites were eluted from the preparative TLC plates with absolute ethanol and concentrated by a stream of air.

A Perkin-Elmer Model 3920B gas chromatograph, which was fitted with a 2-m glass, 3.2 mm I.D. column, packed with 3% Silar 10C on Gas-Chrom Q, (110-200 mesh) (Applied Science Laboratories, State College, PA) was used to detect metabolites not evident by TLC. Two-microliter injections were made at 200°C, and the column temperature was programmed at 70°C for 4 min.; it was then increased 8°C per min. until reaching a final temperature of 160°C, at which point the temperature was maintained for 4 min. The flame-ionization detector was at 200°C, and the nitrogen carrier gas flow rate was 55 ml/min. Ether fractions were subdivided, and 0.1 ml samples were derivatized by adding an equal volume of N, O-bis- (trimethylsilyl) trifluoroacetamide (BSTFA Pierce Chemical Co., Rockford, IL) at room temperature and allowing the material to stand overnight to form trimethylsilyl (TMS) derivatives of polar compounds.

All peaks recovered by GC on Silar 10C column were subjected to electron impact and chemical ionization analysis on a Finnigan 3300 Quadrupole mass spectrometer with Systems Industries 150 Data System. For electron impact, the system was operated at 70 eV; chemical ionization was performed by charging with CH_4.

R_f values for TLC spots were compared to standards of possible metabolites. Standard chemicals were obtained from the following sources: 2,2-dichloroethanol, DCE (Aldrich Chemical Co., Milwaukee, WI), dichloroacetic acid, DCAA (Eastman Organic Chemical, Rochester, NY), dimethyl phosphate, DMP (American Cyanmid Co., Princeton, NJ), monomethyl phosphate, MMP (Stauffer Chemical Co., Richmond, CA).

Inorganic phosphate was detected in a phosphorus-free medium prepared by adding (per liter of distilled deionized water) 0.3g of KCl, 0.2g of $MgSO_4 \cdot 7H_2O$, 0.5g of NH_4NO_3, 0.025g of $CaCl_2 \cdot 2H_2O$, 2.5mg of $FeSO_4 \cdot 7H_2O$, and either 2.42g Tris-base (Sigma Chemical Co., St. Louis, MO) and 0.2M NaOH to adjust to pH 7.16 or 4.74g of Trizma-maleate and 100 ml 0.2 M HCl to adjust to pH 7.18. All glassware was washed in 10% nitric acid prior to use. Dichlorvos (99%) and/or washed Ve cells were added to 25 ml of salts solution in 50 ml-Erlenmeyer flasks. Zero-time treatments were immediately placed in a freezer, and all others were incubated at 29°C±1°C on a rotary shaker. Samples taken at 2 and 4 days were also frozen. At the time of analysis, all samples were thawed and treatments containing Ve cells were centrifuged to remove the cells. Inorganic phosphorus (P_i) determinations were run on the supernatant liquids according to the phosphomolybdate complex formation method described by Strickland and Parsons (1972). The mean and standard deviations of three replicates for each point were recorded.

RESULTS AND DISCUSSION

Figure 1 illustrates a typical analysis from the injection of 2-µl of concentrated, derivatized, neutral ether extract. The neutral extracts contain most of the remainder of the parent dichlorvos molecule; this molecule is not silylated and has a retention time of 13:00 min. By TLC, the R_f of dichlorvos was 0.90. Extraction efficiency was not determined, and neutral extraction did not remove all the dichlorvos from solution; evidence for this was obtained by noting the presence of dichlorvos in acidic extracts tested both by TLC and GLC.

Organic phosphate esters are more soluble in water than in ether or other non-polar solvents. Boush and Matsumura (1967) reported that 83% of ^{14}C-dichlorvos metabolites were found in the aqueous phase after extraction with chloroform. Daughton et al. (1976) concluded that partitioning dialkyl phosphate esters into solvents is inefficient, at best, but enough for analysis can be extracted in this manner. In our study, ether extracts were derivatized to aid in identifying phosphate esters; however, derivatization was incomplete, and hydrolysis of the derivatizing agent, BSTFA, occurred, reducing its usefulness in resolving the identity of these compounds. Peak number 4 in figure 1 is a by-product of BSTFA. Peak number 7 possibly is a doubly silylated derivative of monomethyl phosphate, but this structure was not confirmed. The smallness of the peak may result from its poor partitioning characteristics.

The aqueous phase of the extracted samples was not processed for detection of dialkyl or alkyl phosphates, which are expected products of the cleavage of dichlorvos. In all probability, the dimethyl and/or methyl phosphates will be found there, but this was not shown. However, the aqueous phase was analyzed for the presence of inorganic phosphate, and the results are presented in Table 1.

During the 4-day incubation period in tris-buffered phosphate-free medium, a 6-7 fold increase in inorganic phosphate was detected both in treatments containing Ve and dichlorvos and containing cells alone. A reason for the large difference between the 1st and 3rd treatments at day 0 is the time required to freeze each sample, during which time some additional breakdown occurred because of the heavy inoculum size. Inasmuch as no cell growth was evident by counting organisms on a dichlorvos agar medium, the increase in P_i in the cells-alone treatment is attributable to leakage and release by cell death. In a second experiment, in which a readily utilizable carbon source (maleate) was available, no increase in P_i was found in the cells-alone treatment, whereas the P_i increased almost 9-fold where cells, dichlorvos, and maleate were together. These results suggest a cometabolic pathway for the biological cleavage of dichlorvos. Quantitatively, less than 0.5% of total dichlorvos phosphorus is converted in this manner.

Although the increase of P_i with time in treatment 1 suggests a biological mechanism of cleavage, an abiotic hydrolytic cleavage of dichlorvos cannot be discounted. Methods of detecting alkyl phosphate esters were not

successfully employed in this study. The likelihood of hydrolytic cleavage is suggested by published reports. El Beit (1978) reported chemical cleavage of dimethoate, and Kearney and Helling (1969) noted cleavage of diazinon, both in soil. Faust and Gomaa (1972) showed that organophosphate pesticides could be hydrolyzed at the P-OX bond under alkaline conditions, whereas the PO-X bond is cleaved under acidic conditions. The resultant structure in each case is P-OH. Using tris-buffered salts medium, the pH in the treatments with Ve cells shifted from neutral to alkaline, suggesting the former mechanism as most likely in the experiments of this study.

Evidence of demethylation was also obtained in the analysis. The presence of mono-methoxy phosphates (figure 1, peaks 6 and 7) was suggested in both chemical and biological treatments; however, a positive identification by M.S. was not made. Both biological and chemical demethylation can occur. In mammals, dichlorvos exhibits methylating activity, albeit weak (Wright et al. 1978). Schultz et al. (1971) proposed the formation of desmethyl dichlorvos ($(OH)_2P(O)\ CH{=}CCl_2$) in animal tissues prior to cleavage of the molecule. Bacterial demethylations are known as well, but the bacterial demethylation of dichlorvos has not been shown. In our study, the time of occurrence of demethylation in the pathway of dichlorvos metabolism was not determined. Several low-molecular-weight esters were identified in the biological treatment, but whether these resulted from methylation by methyl groups liberated from dichlorvos or as a result of extraction of bacterial cellular materials is not clear.

Figure 2 shows the results of an analysis of the underivatized acidified extract. Two peaks are of primary interest. The first, peak 3, is dichloroethanol (DCE). The leaving group, $-OCH{=}CCl_2$, likely undergoes reduction and rearrangement of the double bond, yielding the corresponding carbonyl compound, dichloroacetaldehyde ($OCHCHCl_2$) (Eto, 1974; Schultz et al., 1971). The dichloroacetaldehyde may then be reduced to the DCE or possibly oxidized to dichloroacetic acid (DCAA, $HOOCCHCl_2$). The pathway proposed in figure 3 illustrates these reactions. The observation of DCE in treatment 1 suggests an enzymatic mechanism for its formation. DCAA was not found in any treatment by mass spectroscopy, and was not detected on TLC plates.

One other compound, strongly suggested to be 1, 1-dichloroethyl acetate by mass spectroscopy, was also found in the acidic extract (figure 2, peak 1). Inasmuch as dichloroethanol has been shown to be present as well as methyl and ethyl acetate, acetic acid and other acids in the biological treatment 1, conjugation of this alcohol and an acid to form this ester is a possible enzymatic reaction. The spot noted on TLC plates of the acidic extract had an R_f of 0.6; when eluted, it co-chromatographed with this ester.

Thus, a pathway for the degradation of dichlorvos can be proposed. The confirmed presence of DCE and suggested occurrence of a chlorinated ester is evidence of the cleavage of dichlorvos in similar fashion to other organophosphate pesticides, i.e., at the P-OX linkage (X is the chlorinated leaving group).

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Table 1. Formation of inorganic phosphate in the breakdown of dichlorvos (DDVP)

Treatment[a]	Inorganic phosphate-P (ppb)		
	Day 0	Day 2	Day 4
1	24.8±0.25	85.4±3.4	152±0.12
2	ND[b]	ND	ND
3	4.56±0.42	16.8±0.96	31.1±3.6
4	14.5±12.2	1.13±0.63	9.87±7.31

[a]Treatments: 1) DDVP (250 µg/ml) inoculated with bacterial enrichment, Ve.

2) DDVP (250 µg/ml) without inoculation

3) Bacterial enrichment, Ve, in carbon-free medium without DDVP.

4) Bacterial enrichment, Ve, in medium containing maleate but without DDVP.

[b]Not detected.

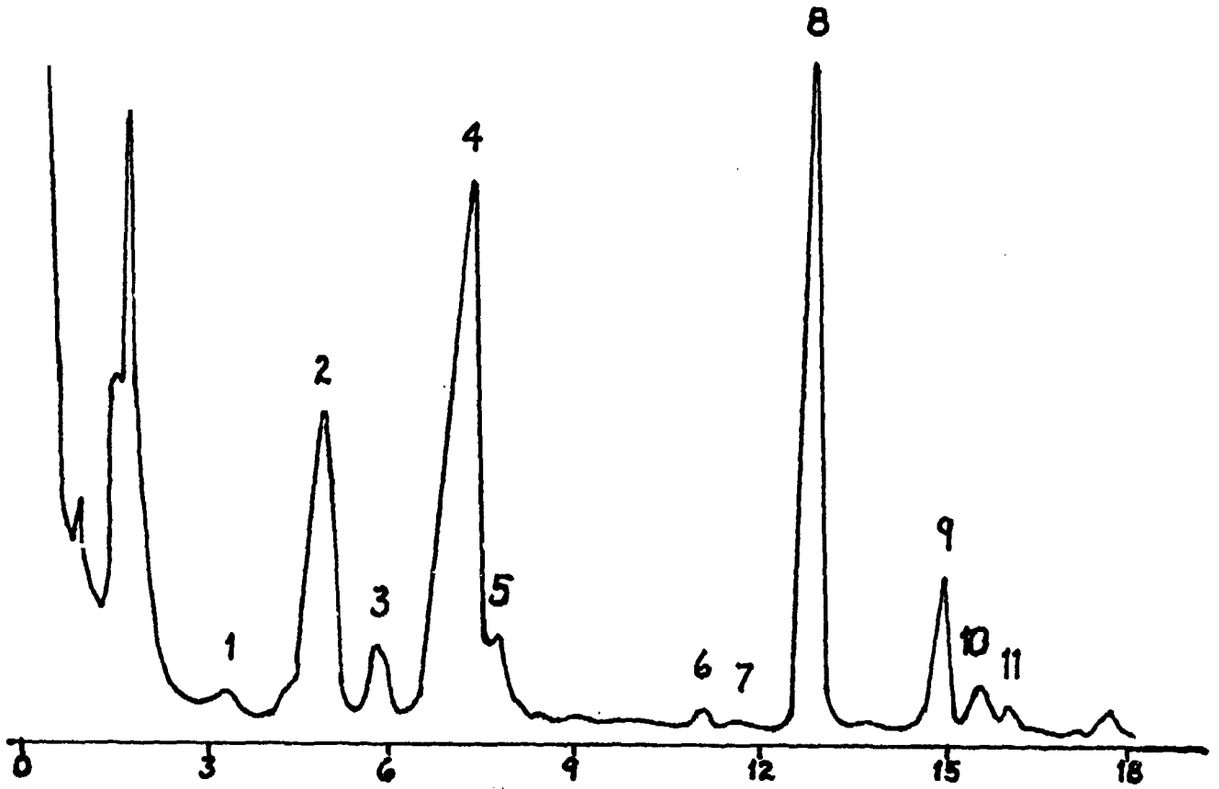

Figure 1. Gas chromatographic analysis of treatment 1 containing 250 µg/ml dichlorvos and Ve cells at neutral pH after ethylether extraction and derivatization with BSTFA (bis (trimethylsilyl)-trifluoroacetamide). Peaks analyzed by mass spectrometry are numbered; retention times are in parenthesis.

1. Not identified; 2) ethylacetate (4:50); 3) organic acid-TMS (5:55); 4) trifluoroacetamide (7:25) from BSTFA; 5) organic acid-TMS (7:50); 6) $^{+}H_4CO\ P(O)(OCH_2CH_3)$ (O-TMS H^+) ? (11:15); 7) $H_3CO\ P(O)\ (O\text{-}TMS)_2$? (11:50); 8) dichlorvos (13:00); 9, 10, and 11 not identified.

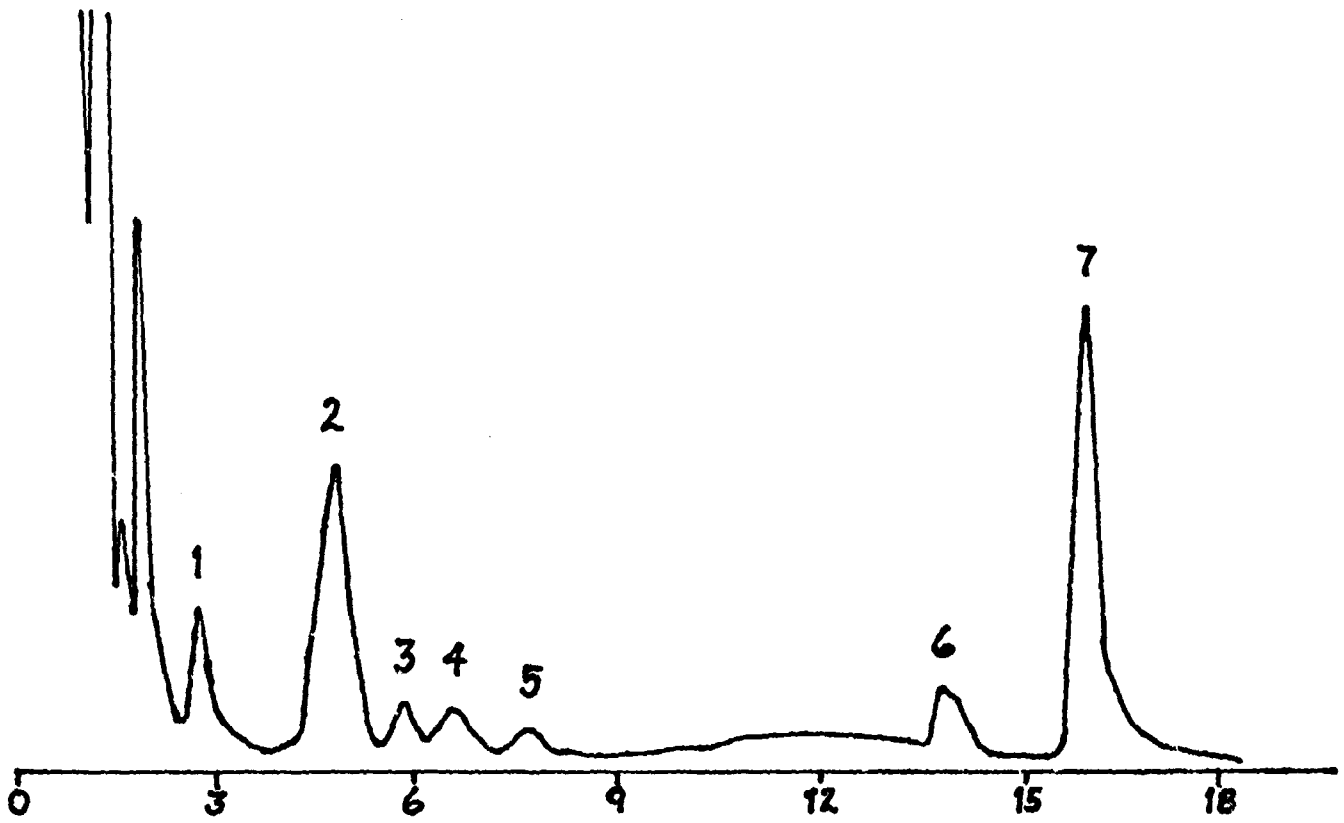

Figure 2. Gas chromatographic analysis of treatment 1 containing 250 µg/ml dichlorvos and Ve cells after acidification and ethylether extraction. Peaks analyzed by mass spectrometry are numbered; retention times are in parenthesis. 1) 1,1-dichloroethyl acetate (2:45); 2) ethyl acetate (4:50); 3) 2,2-dichloroethanol (5:45); 4) methyl acetate (6:33); 5) acetic acid (7:40); 6) (13:45) and 7) (16:00) not identified.

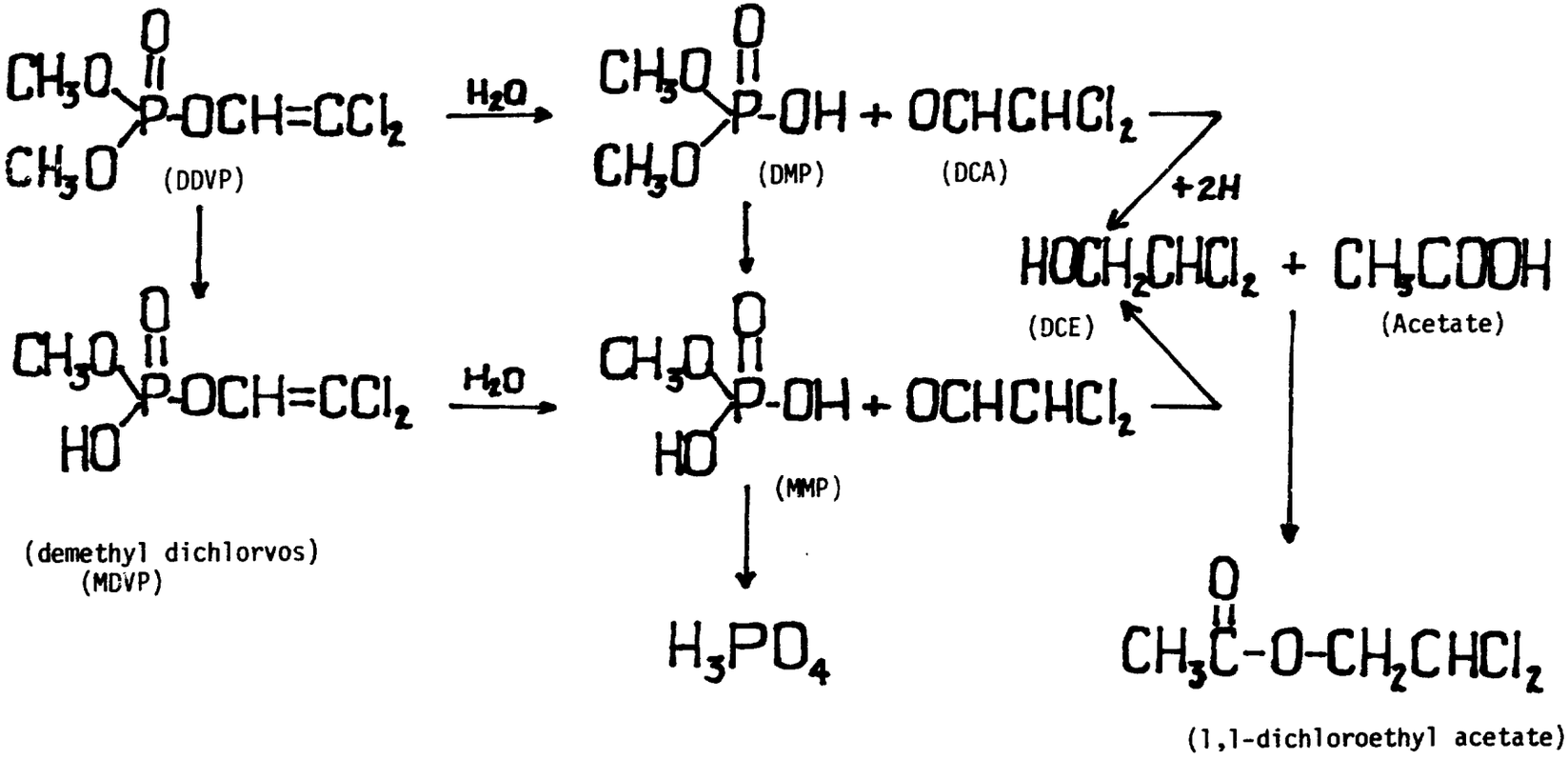

Figure 3. Proposed pathway for the breakdown of dichlorvos.

TECHNICAL REPORTS

The only technical reports issued were the annual reports submitted in 1978, 1979 and 1980. They are entitled, "Microbial degradation of pesticides" and have Defense Documentation Center numbers ADA047675, ADA061887 and ADA078456.

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Rosenberg, A., and M. Alexander. 1980. Microbial metabolism of 2,4,5-trichlorophenoxyacetic acid in soil, soil suspensions, and axenic culture. J. Agric. Food Chem. 28:297-302.

Rosenberg, A., and M. Alexander. 1980. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) decomposition in tropical soil and its cometabolism by bacteria in vitro. J. Agric. Food Chem. 28:705-709.

Lieberman, M. T., and M. Alexander. 1981. Effects of pesticides on decomposition of organic matter and nitrification in sewage. Bull. Environ. Contam. Toxicol. 26:554-560.

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